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Analysis of Barbiturates by GC/MS, HPLC and Chemical Ionization

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BY

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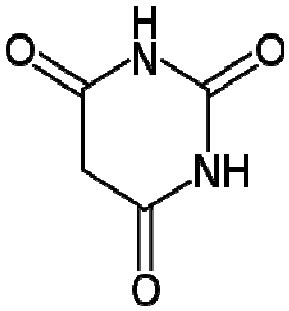
ABSTRACT:

Barbiturates are derivatives of barbituric acid. They act as central nervous depressants. These drugs are frequently used as sedatives or anesthetics. It is a simple and reproducible method for the analysis of barbiturates.

Usage of these drugs is increased in recent days and the analysis of barbiturates by GC/MS is very fast. As they are used as sedatives their usage has been increased to relieve the stress. But the over usage of these drugs may lead to death. Different barbiturates like phenobarbital, pentobarbital can be analyzed by their molecular weights. Because of the over usage of the drugs the interest to analyze them has been enhanced in order to know the characteristics.

INTRODUCTION:

The purpose of this study is to analyze the barbiturates from the mixture by using GC/MS and HPLC. Barbiturates are intially derived from urea.



Barbiturate mixture contains different barbiturates such as Amobarbital, Pentobarbital, Butabarbital, Phenobarbital, Secobarbital. By using their CAS numbers and from the library search their molecular weights and structures were found.

Separation and analysis of barbiturates from mixture is done bye using GC/MS. The sample is also run through HPLC to know the least concentration at which the separation can be done.

Chemical Ionization(CI) is performed to know fragment ion peaks. Ions are produced through the collision of the analyte with ions of a reagent gas that are present in the ion source. Some common reagent gases are ammonia, isobutene, methane. It is a low energy process.

S NO	NAME OF BARBITURATE	MOLECULAR WEIGHT	CAS NUMBER	MOLECULAR FORMULA
1	Amobarbital	226	57-43-2	$C_{11}H_{18}N_2O_3$
2	Pentobarbital	226	76-74-4	$C_{11}H_{18}N_2O_3$
3	Butabarbital	224	77-26-9	$C_{11}H_{16}N_2O_3$
4	Phenobarbital	232	50-06-6	$C_{12}H_{12}N_2O_3$
5	Secobarbital	238	29071-21-4	$C_{12}H_{18}N_2O_3$

EXPERIMENT:

CHEMICALS USED:

Barbiturates mixture
Methanol
Water
Methylene chloride.
Sample was initially dissolved in the 0.5ml of methylene chloride and this solution was used for the analysis. Different concentration of solution was injected and the resolutions were observed

Instrumentation:

Software used was chemstation and Brukers software.

GC/MS:

- Injection port temperature: 200° C
- Total run time: 25min

- Scan range: 15-300m/z
- Split ratio: 10/1

Column Oven:

Temperature	Rate	Hold time	Total time
100	-	0.00	0.00
300	8.0	0.00	25.00

HPLC:

- Solvents: A: water
  B: Methanol
- Flow rate: 0.50µl
- Column used: C18
- Signal: DAD

Gradient Table

Time	B%
0.00	30
1.000	50
2.00	70
3.00	90

METHOD:

Software used was chemstation and brukers.

Sample was intially dissolved in the 0.5ml of methylene chloride and this solution was used for the analysis. Different concentration of solution was injected and the resolutions were. Observed Mixture consists of different types of barbiturates such as amobarbital, phenobarbital, pentobarbital, butabarbital, secobarbital.

when the split was off resolution was found to be good. Initially solvent cut was 1.5 and this was changed to 2.0. Amount sample injected was varied between 1-2µl. Total run was for 25minutes.

Molecular weights and fragments were obtained initially from the library search by their cas numbers.

Peaks obtained when the sample was injected were scanned seperately and then compared with standards obtained from library search.

RESULTS AND DISCUSSIONS:

Results of GC/MS:

When the sample was run in GC/MS we observed five different peaks by scaning the each peak separately we observed different fragment ion peaks of different barbiturates.

PEAK	RETENTION TIME (min)
1	9.044
2	9.798
3	10.767
4	11.681
5	12.968

By scanning the peak 1 we observed the fragment ion peaks at 212.9, 211.

Scanning of second peak gives peaks at 227, 227.8 may be amobarbital or pentobarbital.

Scanning of third peak gives fragments at 239.2, 238.2 may be secobarbital.

Scanning of fourth peak gives fragment ion peaks at 221.5, 220.6 may be butabarbital.

Scanning of fifth peak gives the fragment ion peaks at 233.2 which may be phenobarbital.

According to the graphs obtained we can observe the good separation of the compounds in GCMS. The results of LCMS were not good, Resolution was not clear. The compounds were not separated properly.

RESULTS OF LC/MS:

For LC/MS the reolution was found but it was not proper. When the peaks were scanned then the fragments were observed at 250 and 280 where we didn't found any barbiturates.

DISCUSSION:

According to the graphs obtained we can observe the good separation of the compounds in GCMS. The results of LCMS were not good, Resolution was not clear. The compounds were not separated properly.

Derivatization of the sample before injection may give good resolution for HPLC. Derivatization eliminates the interference. Resolution and retention times can be improved. The UV detection of barbiturates can be significantly enhanced through post column photochemical derivatization. Barbiturates have no significant absorbance above 230 nm and detection in the 200–220-nm range can be complicated by the presence of interfering peaks. A Fluorescence-generating reagents are used.

CONCLUSION:

By observing the results of GC/MS the resolution was found to be very good but for the HPLC the resolution was not proper. Pre derivatization of the sample before injection may give good resolution.

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